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Confinement Behavior of Cape Honey Bees (*Apis mellifera capensis* Esch.) in Relation to Population Densities of Small Hive Beetles (*Aethina tumida* Murray)

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We quantified the effects of increasing small hive beetle (Aethina tumida Murray) populations on guarding behavior of Cape honey bees (Apis mellifera capensis, an African subspecies). We found more confinement sites (prisons) at the higher (50 beetles per colony) rather than lower (25 beetles per colony) beetle density. The number of beetles per prison did not change with beetle density. There were more guard bees per beetle during evening than morning. Neither guard bee nor beetle behavior varied with beetle density or over time. Forty-six percent of all beetles were found among the combs at the low beetle density and this increased to 58% at the higher one. In neither instance were beetles causing depredation to host colonies. Within the limits of the experiment, guarding behavior of Cape honey bees is relatively unaffected by increasing beetle density (even if significant proportions of beetles reach the combs).

KEY WORDS: confinement; *Apis mellifera capensis*; *Aethina tumida*; *A. tumida* behavior; guard bee behavior; small hive beetles.

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INTRODUCTION

The initial defense used by host colonies of Cape (*Apis mellifera capensis* Esch, an African subspecies of honey bee) and European honey bees (*A.m.* L.) against invading small hive beetles (*Aethina tumida* Murray) is a confinement scheme where beetle movement is restricted by guard bees who keep the beetles detained in cracks and crevices throughout the colony (Hepburn and Radloff, 1998; Neumann *et al.*, 2001; Ellis *et al.*, 2003a,b). In an attempt to explain European bee susceptibility and Cape bee immunity to depredation caused by beetles, initial studies suggested that confinement schemes of European bees might be less efficacious than those of Cape ones (Neumann *et al.* 2001; Solbrig, 2001; Ellis *et al.*, 2003a). However, recent evidence suggests that at low intracolonial beetle densities, confinement behaviors of Cape and European honey bees do not differ significantly (Ellis *et al.*, 2004).

Despite similarities in fundamental confinement behaviors of Cape and European honey bees, Cape bees may handle increasing, intracolonial beetle populations differently from their European counterparts. Here we report the effects of increasing beetle density on beetle confinement and guarding behavior of Cape bees. The data allow for comparisons to be made between confinement schemes of Cape and European honey bees and ultimately place the efficacy of these behaviors as resistance mechanisms to beetles in context.

MATERIALS AND METHODS

Experiments were conducted in Grahamstown, South Africa (February–March 2003) following procedures established in earlier work by our lab (Ellis *et al.*, 2003a,b). Three observation hives were used each containing two frames of brood, one of honey, about 8000 bees, and a laying queen (all unrelated). All bees, combs, and queens were from established colonies of Cape honey bees and in a geographic region where beetles commonly occur. A transparent grid, which divided each side of the colony into 160 squares (5 cm $\times$ 5 cm each), was used to define intracolonial locations that consisted of the top wall (above the uppermost frame), bottom board, front wall, back wall, and rest (among the combs) of the colony.

Twenty-five, randomly-collected beetles (to minimize the possibility of sex-specific behaviors biasing the results) were introduced into two of the colonies and 15 days later, the colonies were monitored twice daily at approximately 08:00 and 20:00 hours (under red-light conditions) for 3 days. On the fourth day of observations, 25 more beetles were added to both colonies (raising the beetle population to about 50 beetles per hive as most beetles from the first introduction were still in the hive) and on days 5–7, the colonies were monitored again. For the third colony, a procedure similar to that described above was conducted, except initial monitoring began 1 day after the introduction of beetles into the colony. At each monitoring interval, the observer moved across the top row of the grid, from left to right, and then down one block (or one 5-cm^2 area) in the grid, followed by another left to right motion. This pattern was followed from top to bottom on both sides of the hive. Neither beetles nor bees were counted twice in any observation because guard bees and beetles do not readily move between prison areas. The entire procedure lasted approximately 30 min per hive.

Intracolonial distribution, behavior, and number of imprisoned beetles, and number and behavior of worker honey bee guards (guarding at prison entrances) were recorded. Beetle behavior included resting, mating, and antennal or trophallactic contact with guard bees. Guard bee behavior included biting, antennating, and trophallactically feeding beetles, and prison wall-working (all behaviors have been previously described for Cape and European honey bees: Neumann *et al.*, 2001; Solbrig, 2001; Ellis *et al.*, 2003a).

Guard bee and beetle behaviors and prison dynamic variables were analyzed with a repeated measure ANOVA design recognizing beetle density (25 or 50 beetles) and time (morning or evening) as main effects. Because data for guard bee and beetle behaviors were proportions, the data were transformed using arcsin $\sqrt{}$ proportion to stabilize the variance. Encapsulated beetle intracolonial distribution was analyzed by beetle density using Pearson's χ^2 tests. Significant differences were accepted at the ≤ 0.05 and all analyses were conducted using Statistica (2001).

RESULTS

The number of beetle prisons per colony was significantly affected by beetle density and time (Table I) with there being more prisons at the higher beetle density than at the lower one (Table II) and during evening than morning (Table III).

Although the number of prisons increased, the number of beetles per prison did not increase at either beetle density or change in time (Tables II and III). The number of guard bees per encapsulated beetle increased from morning to evening (Table III) but did not significantly differ over beetle density (Table II). Further, the number of guard bees per prison was not affected by time or beetle density (Table I).

Table I. Analysis of Variance Testing Effects of Beetle Density (d), Time (t), and Time$\times$ Density ($t \times d$) on Confinement Dynamics, Beetle Behavior, and Guard Bee Behavior

Variable	Source	df	F	$P > F$
Confinement dynamics				
Number of guard bees per beetle	d	1	0.1	0.7897
	t	1	13.4	0.0021
	$t \times d$	16	3.9	0.0665
Number of prisons per colony	d	1	19.3	0.0005
	t	1	8.9	0.0087
	$t \times d$	16	0.2	0.6598
Number of beetles per prison	d	1	0.3	0.6002
	t	1	0.7	0.4126
	$t \times d$	16	0.0	0.9834
Number of guard bees per prison	d	1	0.9	0.3519
	t	1	4.3	0.0537
	$t \times d$	16	1.6	0.2175
Beetle behavior				
Resting	d	1	0.1	0.7342
	t	1	0.4	0.5177
	$t \times d$	16	1.5	0.2402
Making antennal contact with guard bees	d	1	0.2	0.6766
	t	1	2.6	0.1246
	$t \times d$	16	0.0	0.9356
Getting fed by guard bees	d	1	0.0	0.9509
	t	1	0.0	0.9281
	$t \times d$	16	3.8	0.0692
Mating	d	1	0.5	0.4786
	t	1	0.4	0.5548
	$t \times d$	16	0.5	0.4703
Guard bee behavior				
Biting at beetles	d	1	0.0	0.8683
	t	1	0.0	0.8272
	$t \times d$	16	1.5	0.2360
Making antennal contact with beetles	d	1	0.3	0.5678
	t	1	0.3	0.5956
	$t \times d$	16	1.1	0.2999
Feeding beetles	d	1	1.1	0.3097
	t	1	0.4	0.5140
	$t \times d$	16	3.8	0.0683
Prison wall-working	d	1	0.0	0.8748
	t	1	0.1	0.7363
	$t \times d$	16	2.1	0.1661

Beetle activity did not increase at the higher beetle density (Table II) or either time (Table III). Additionally, time and beetle density did not significantly affect the proportion of beetles making antennal contact with guard bees, getting fed by guard bees, or mating. Further, none of the measured behaviors of guard bees (biting at, making antennal contact with, and feeding beetles and prison wall working) were affected by time or beetle density (Tables I, II, and III).

Table II. Small Hive Beetle Density Effects on Confinement Dynamics, Beetle Behavior, and Guard Bee Behavior

	25 beetles Mean ± SE	50 beetles Mean ± SE
Confinement dynamics		
Number of guard bees per encapsulated beetle	0.98 ± 0.10a	1.02 ± 0.09a
Number of beetle prisons per colony	7.94 ± 0.63a	14.17 ± 0.98b
Number of beetles per prison	2.46 ± 0.19a	2.74 ± 0.34a
Number of guard bees per prison	2.25 ± 0.20a	2.60 ± 0.28a
Beetle behavior		
Resting	0.82 ± 0.03a	0.86 ± 0.02a
Making antennal contact with guard bees	0.07 ± 0.02a	0.05 ± 0.01a
Getting fed by guard bees	0.02 ± 0.01a	0.01 ± 0.01a
Mating	0.02 ± 0.01a	0.01 ± 0.00a
Guard bee behavior		
Biting at beetles	0.58 ± 0.05a	0.58 ± 0.04a
Antennal contact with encapsulated beetles	0.07 ± 0.02a	0.05 ± 0.01a
Feeding encapsulated beetles	0.02 ± 0.01a	0.02 ± 0.01a
Prison wall-working	0.07 ± 0.03a	0.06 ± 0.02a

Note. For beetle and guard bee behavior, data are the proportion of individuals observed doing the particular behavior. $n = 9$ for all data. Row totals followed by the same letter are not different at the $\alpha \leq 0.05$ level. Means were compared using ANOVAs.

There was a significant effect of beetle density on intracolonial beetle distribution ($\chi^2 = 14.9$; df $= 4$; $P = 0.0049$). The proportions of beetles found on the bottom board, front wall, and back wall of the hive all decreased at the higher beetle density leading to a marked increase of beetles among the combs at the higher density (Table IV). Despite the high percentage of beetles found among the combs at both beetle densities, most (>90% based on visual estimations) of the beetles reaching the combs were kept out of the brood, honey, and pollen areas by bee aggression and were instead confined to empty cells around the comb periphery.

DISCUSSION

In our earlier work on beetle confinement by European bees, we showed increasing beetle density led to more confinement sites (prisons); beetle density per prison did not change (Ellis *et al.*, 2003b). Our data show the same trends for Cape honey bee colonies. This could mean that there are optimum beetle densities per prison most efficiently guarded by bees or that beetles disperse evenly throughout the colony and are confined wherever they hide. We further found the number of prisons increased during evening, perhaps indicating a more general increase in beetle dispersal during evening.

Table III. Time (Morning and Evening) Effects on Confinement Dynamics, Beetle Behavior, and Guard Bee Behavior

	Morning Mean ± SE	Evening Mean ± SE
Confinement dynamics		
Number of guard bees per encapsulated beetle	0.84 ± 0.06a	1.16 ± 0.10b
Number of beetle prisons per colony	9.94 ± 0.94a	12.17 ± 1.21b
Number of beetles per prison	2.68 ± 0.26a	2.51 ± 0.29a
Number of guard bees per prison	2.11 ± 0.17a	2.74 ± 0.29a
Beetle behavior		
Resting	0.85 ± 0.02a	0.83 ± 0.03a
Making antennal contact with guard bees	0.04 ± 0.01a	0.08 ± 0.02a
Getting fed by guard bees	0.02 ± 0.01a	0.01 ± 0.01a
Mating	0.02 ± 0.01a	0.01 ± 0.01a
Guard bee behavior		
Biting at beetles	0.56 ± 0.06a	0.59 ± 0.04a
Antennal contact with encapsulated beetles	0.05 ± 0.02a	0.06 ± 0.01a
Feeding encapsulated beetles	0.02 ± 0.01a	0.02 ± 0.01a
Prison wall-working	0.07 ± 0.03a	0.06 ± 0.02a

Note. For beetle and guard bee behavior, data are the proportion of individuals observed doing the particular behavior. $n = 18$ for all data. Row totals followed by the same letter are not different at the $\alpha \leq 0.05$ level. Means were compared using ANOVAs.

Why the number of guard bees per beetle did not increase with increasing beetle density in Cape colonies as it did in European colonies (Ellis *et al.*, 2003b) is unclear; however, it may be due to the absence of increasing beetle activity at the higher density and evening. Because beetle activity did not increase, more guards were not needed to keep the beetles confined. Other studies (Ellis *et al.*, 2003a,b) have shown a positive correlation between the level of beetle activity and the number of guard bees. The lack of increasing beetle activity at the higher density and evening may indicate that Cape bees were able to keep beetle activity low. Indeed, beetle activity in this study was lower than that found for beetle activity in European colonies (21% and 39% of beetles were active in European colonies at 25 and 50 beetles per colony, respectively, Ellis *et al.*, 2003b).

Table IV. Proportion of Small Hive Beetles Encapsulated in Various Intracolonial Locations at Both Beetle Densities

Location	25 beetles Mean ± SE, $n = 18$	50 beetles Mean ± SE, $n = 18$
Top wall of hive	0.04 ± 0.02	0.05 ± 0.01
Bottom board of hive	0.24 ± 0.04	0.20 ± 0.04
Front wall of hive	0.14 ± 0.04	0.10 ± 0.03
Back wall of hive	0.12 ± 0.02	0.08 ± 0.02
Combs	0.46 ± 0.07	0.58 ± 0.07

We found that beetle behavior in Cape bee colonies remained fairly consistent over beetle density and time. Further, guard bee behavior remained relatively unaffected by beetle density or time, unlike that found for the behavior of guard bees in European colonies, which significantly changed at the higher beetle density (Ellis *et al.*, 2003b). This suggests that Cape bees are better able to handle changing beetle density than are European bees, or at least that their confinement behavior is more consistent through changing beetle density than that of European bees.

Perhaps most significant are our findings concerning intracolonial beetle distribution. In earlier work on beetle confinement by Cape bees, we found that as much as 23% of beetles in a colony can be found among the combs (Ellis *et al.*, 2004). In this study, we found 46% of beetles at the lower density and 58% of beetles at the higher density among the combs. These percentages are much higher than those reported from European colonies (Ellis *et al.*, 2003a,b). Although over half of the beetles managed to reach the combs in the present study, few accessed bee brood, honey, or pollen and this may be due to general bee aggression. Indeed, African bees are significantly more aggressive toward free-roaming beetles than their European counterparts (Elzen *et al.*, 2001).

These findings strongly suggest that confinement of beetles is not the sole mechanism by which Cape bees limit depredation caused by beetles because a large proportion of beetles gained access to the combs where they can reproduce. Although fundamental confinement behaviors of Cape and European bees are similar, we have shown that once beetle density in a colony increases, both bee subspecies handle the increase differently. Increasing beetle density did not significantly alter confinement behavior by Cape bees.

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